

1/12/2004

CLAIMS AMENDMENT

What is claimed is:

- 112 f2 1. The use of thiophosphate as a substitute for normal phosphate in microbial cultures to create nucleic acids in vivo modified with phosphorothioate internucleotide linkages.
2. The method of claim 1 used to generate phosphorothioate ds DNA, ss DNA, and/or RNA by the in vivo incorporation of thio-phosphate into nucleotide precursor pools.
3. The method of claim 1 used to generate ds DNA, ss DNA, or RNA partially substituted with phosphorothioate linkages by culturing cells in media consisting of the in vivo incorporation of a mixture of thio-phosphate and inorganic phosphate into nucleotide precursor pools.
4. The method of claim 1 wherein the cells cultured in thio-phosphate media or induced to uptake thio-phosphate are of eukaryotic origin.
- 112 f2 5. The method of claim 1 wherein other derivatives instead of thio-phosphate another phosphate derivative is used including a phosphate where the oxygen atoms are replaced with one two or more sulfur groups, one or more methyl groups, or any other moiety, or any combination of alternate moieties and

incorporated in vivo into nucleotide precursor pools.

6. A method for creating phosphorothioate oligos in vitro comprising:

- 1) isolating recombinant phage, plasmid, or other nucleic acid modified in vivo with phosphorothioate linkages
- 2) purifying the sequences corresponding to the recombinant nucleic acid insert
- 3) fragmenting the nucleic acid insert with DNase I, S1 nuclease, or other means creating an overlapping set of oligo mixtures spanning the entire length of the segment.